SHORT COMMUNICATION

Serum sialic acid alterations in the hamster cheek pouch carcinogenesis

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The hamster cheek pouch mucosal carcinoma model, as developed by Salley1), is an excellent experimental system for the study of oral mucous membrane malignancy. The development of tumors is so consistent, though past investigations were based mainly on morphologic studies, that the time dependent biochemical changes may be identified. Recently, attention has been focused on the surface protein of cancer cells, including tumor associated antigens, which might distinguish them from normal cells. Some glycoproteins and glycolipids, which are constituents of the cell surface, are important to cancer related properties, and sialic acid is a common terminal saccharide of these glycoproteins and glycolipids2). A neoplasm often has an increased concentration of sialic acid on the tumor cell surface, and sialoglycoproteins are shed or secreted by some of these cells, which increases their concentration in the blood3, 4).

In this report, we describe the progressive changes of sialic acid concentration in the sera of the hamsters submitted to the carcinogenesis experiment to appraise the significance of the value as a "tumor marker".

In addition, the jugular venipuncture5), which enables us to obtain blood up to 1 ml/once a week for 20 weeks from the same hamster, is recommended and the feasibility of this method is confirmed in the present study.

Materials and Methods

Male Sylian golden hamsters, 8 weeks of age and weighing 90 g, were housed in cages individually, fed a solid laboratory diet, and given tap water ad libitum. Four unanesthetized animals received three applications per week to the left cheek pouch of 0.3 % (W/V) 9, 10-dimethyl-1, 2-benzanthracene (DMBA) in acetone for 14 weeks by means of #1 raccoon dog's hair brush. The application of DMBA was performed on the posterior one-third of the medial wall of the cheek pouch as localized as possible. The animals with growths in their treated cheek pouches received no further treatment during the remaining 7 weeks of the experiment as described in the previous communication6). Five hamsters of the same age, and sex, but which received no treatment during the whole experimental period (20 weeks), were used for comparison.

Serum analysis: blood samples, 1 ml/once a week, from control and DMBA-treated animals, were obtained by the method described in the previous communication5) under ether inhalation anesthesia. After clotting for 1-2 hours at room temperature, samples were kept at 4°C overnight. The sera were separated by centrifugation of the blood samples at 1,000×g, for 10 min, and stored at −20°C until used.

Total sialic acid concentration in serum
was measured by the enzymatic method of Araki\textsuperscript{7)}, Kyokuto Pharm. Ind. Co. LTD.. Student's $t$-test was used to determine if there was a difference in the mean values for sialic acid between the tumor bearing group and normal control group.

**Results**

The tissue reactions of the hamster cheek pouches against DMBA, with the concentration and schedule of painting employed in this experiment, were divided into four main sequences. The sequence of reactions described below were almost the same as that of other investigators\textsuperscript{8}) while light and electron microscopic observations were reported in our previous communication\textsuperscript{9}). In the first to second week (Period I), inflammation in the treated cheek pouches commenced after 2 or 3 applications of DMBA. The cheek pouch exhibited marked edema and surface ulcerations. In the 3rd to 9th week (Period II), the cheek pouch appeared thickened and grayish white, uniformly following subsidence of inflammation in spite of continuation of DMBA painting. Small papillomatous growths were formed at about 10 weeks. In the 10th to 13th week (Period III), the growths grossly increased in size. After approximately 14 weeks (Period IV), one or more tumors 1 cm or more in diameter were present and continued to grow at the 20th week in spite of the cessation of painting at the 14th week.

The relationship between serum sialic acid concentration and time course of carcinogenesis is shown in Fig. 1. Sialic acid levels in the serum of control hamsters remained constant irrespective of their age (8 to 28 weeks) with small experimental variations (17.69 ± 1.22 mg/dl, mean ± S.D.). In remarkable contrast, the serum sialic acid content in hamster treated with DMBA began to increase promptly after three applications of carcinogen but showed a prompt decrease into normal levels at the 4th week in spite of continuing DMBA treatment. This alteration correlated with that of Period I, in which the inflammatory reaction could be observed in the cheek pouch. Sialic acid concentrations kept a normal range from the 4th to the 9 or 10th week (Period II). Sialic acid concentration showed a tendency to increase again coincident with the tumor appearance at around 10 weeks (Period III), though it was not significant statistically ($p>0.05$). The sialic acid concentration remained slightly elevated even though the painting was stopped at the 14th week and showed a significant difference compared with the control group ($p<0.01$) from 16th to 20th week (Fig. 1).

In order to consider the origin of the sialic acid in the serum the concentration of sialic acid in the tumors, a mixture of four materials which were obtained at the 20th week, was determined. The concentration of sialic acid (mg/mg proteins) in the tumor tissue of the carcinoma was extremely high, 0.333 mg/mg protein, compared with that of the cheek pouch of control animals, 0.198 mg/mg protein.

**Discussion**

Current data demonstrates that sialic acid content in sera are elevated in all tumor-bearing hamsters from the 16th to 20th week. It is obvious that the increased levels of sialic acid is independent of the age of animals because the values do not vary with the aging of the control group for a period of 8 to 28 weeks. It is interesting to note that this increase of sialic acid content seems to be correlated with the time in which the tumors in the cheek pouch develop to be carcinoma morphologically. Furthermore a slight upward trend is observed since Period III, when papilloma develop and shows gradual growth. The high concentration of sialic acid in serum is also observed from the 1st to 3rd week, which is strongly correlated with the inflammatory stage of carcinogenesis, observed as edema and ulceration on the cheek pouch mucosa macroscopically.

The serum sialic acid levels are well correlated with tumor burden in hamster cheek pouch carcinogenesis even though no such correlation is found in our preliminary experiment of so called "tumor markers" such as carcinoembryonic antigen (CEA), alpha-fetoproteins, tumor antigen (Ta-4), ferritin, immunosuppressive acidic protein (IAP), tissue polypeptide antigen (TPA), and carbohy-
Fig. 1 Progressive changes of sialic acid concentration in serum of the hamsters at the time indicated after initiation of painting with DMBA. The filled symbols and solid lines (—) indicate the experimental group. The open symbols and broken lines (— — —) indicate the control group. Vertical thin lines on each symbol indicate standard deviation (S. D.).

Although serum sialic acid concentrations are often increased in sera of patients with cancer\(^2\), and tumor-bearing rodents\(^3\), the causes of the elevation of sialic acid in sera are unknown. Consequently, the precise significance of the elevated levels of sialic acid in hamsters with carcinoma in their cheek pouches is not known. Some tumors have been reported to have elevated sialic acid contents, including human tumors of the colon, stomach, and breast\(^1\), and experimental tumors of the rat\(^18\). Furthermore, tumors can shed sialic acid containing components into their environment, which increases the concentration in the blood\(^3,4\). Although elevated sialic acid contents in the tumors yielded was observed, we can not draw any conclusions from the data, because the values consists of both sialic acid in tumor cells and that of connective tissues which show inflammatory cell infiltration. As for inflammation, a strong correlation is pointed out between the acute phase proteins and the concentration of sialic acid by Turner\(^14\). In fact, elevated sialic acid levels are found in inflammatory diseases and rheumatoid arthritis\(^15\). In this respect, the statistically significant elevations of serum sialic acid are observed in all of the animals in Period I, i.e., inflammatory stage of carcinogenesis, as well as in Period IV. Thus it seems most probable that increased levels of circulating sialic acids may be a secondary response to the tumor.

Regardless of the mechanisms, elevated sialic acid levels emerges as a consistent feature in hamster cheek pouch carcinogenesis. Thus, the determination of sialic acid concentration in serum provides valuable information in the detection of early stage and frank
carcinoma stage. These findings prompt us to investigate the biochemical events in carcinogenesis. Other correlations, such as size of primary tumor or degree of metastasis in combination with other parameters, will give us insight into the biological nature of hamster cheek pouch carcinoma, and consequently into oral cancer in humans.

The data also shows that the jugular venipuncture employed in this study to obtain blood samples is repeatable for a long period of time - 20 weeks - and gives us a large volume (1 ml) of blood each time, from the same hamster. This procedure makes it possible to determine biochemical values from one sample and to observe the biphasic changes of sialic acid concentration in serum in the same animal.

We conclude that the jugular vein technique combined with the monitoring of sialic acid is useful for biochemical elucidation of chemically induced carcinoma in hamsters.

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References